

Appendix 2

Fabrication and investigation of nanometer sized dielectric interdigitated transducers for detection of biomolecular binding

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1. Abstract

A novel sensor concept for sensitive and selective detection of biomolecules is presented. Interdigitated electrodes working as molecular capacitor were fabricated using photo- and e-beam lithography in micro- and nanometer dimensions. The gold electrodes have been modified with self assembling of thiolic molecules carrying a binding site for complex formation. Thus, the binding of relevant biomolecules to the electrode surface can be measured as dielectric response by impedance spectroscopy.

2. Introduction

The microsystem technology opened new fields by the investigation of biochemical and molecular interactions. The transducer and actuators can be miniaturized down to few nanometers to influence or to observe molecular reactions [1,2]. Also miniaturized transducer are usable for the highly sensitive and selective detection of biochemical species in solutions, where novel effects by change of diffusion conditions are employed. A important significance as electrochemical transducers receives the interdigitated array electrodes. The variation and decrease of their electrode dimension from milli- to nanometer lead to novel effects and applications.

Sensors for conductivity measurements with millimeter dimensions are well established in chemical and environmental analysis [3]. The effect of redox recycling, resulting from overlapping of the diffusion layers, can be observed in the range of view micrometer [4-6]. This effect can be used for highly sensitive amperometric measurement of reversible species, e.g. for neurotransmitter [7,8] or for immunosensors [9].

Reducing the distances between two adjacent electrodes, a proportional increase of the electric field strength by an applied bias voltage can be observed. This electric field strength reaches into the MV/m range when the spacing is few micrometers or nanometers. Bias voltages between of 10 - 100 mV became necessary, which avoided regions of oxygen reduction and hydrogen evolution. Such high electric fields are useable for investigation of liquid dielectric media. In liquids it is possible to measure molecular interactions of the changing the rotational motion. Particularly for dipolar molecules lead to a decrease of the permittivity then ac fields are applied [10]. In chemically reactive systems, where the polarity of the reaction partners and

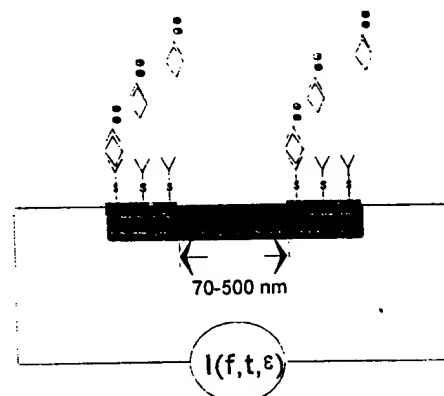


Fig. 1 Scheme of the measuring principle of the DIT as molecular capacitor

the products differ a high electric field can lead to a displacement. The displacement results in changing of the permittivity and can be used for biosensors with enzymatic reactions [11]. Molecular interactions can be measured directly, if the corresponding species influence the dielectric space between the interdigitated electrode pairs. This influence can be realized by aimed binding of the species on the functionalized electrode surface (Fig.1), [12]. Additionally, a strong electric field lead to a polarization of fluctuational regions of a system, if molecular interactions occurs. Then an increase of the permittivity, as the function of electric field, is observable.

This molecular capacitor function we demonstrate on dielectric interdigitated transducer (DIT) for the direct detection of affinity binding without labeling. The DIT is sensitive to the binding of relevant biomolecules on the sensor surface and used the dielectric response as detectable parameter.

As DIT we used gold electrodes with widths and gaps between 50 nm and 4 μm . The electrodes were modified with a self assembled thiol-biotin layer. The biotin has a strong binding with avidin, the couple is commonly used for immobilization of reagents on surfaces. For that reason a avidin-ferritin complex is bounded at the biotin layer. Ferritin has a high dipole moment to influence the capacitor, so that avidin-ferritin served as binding biomolecule to follow the capacitor characteristics in combination with impedance measurement.

3. Experimental

3.1. Electrode Fabrication

Two types of electrodes were used, a multichannel electrode with 16 pairs of interdigitated electrodes, fabricated with standard photolithographic techniques [13] and a DIT structured with e-beam lithography in nanometer scale.

The interdigitated pairs of the multichannel electrode chip were varied in their parameters of electrode width and gap between 800 nm and 4 μm , in order to obtain a uniform modification for different capacitance arrangements.

For the DIT which were structured in the nanometer scale, the e-beam lithography procedure is described in detail. Polymethylmethacrylate (PMMA) was exposed in a Philips/ Leica EBPG-4HR electron beam system as key process step supplemented by galvano-plating for producing the metal electrodes. Standard 4 in. silicon wafers were used as substrate material on which a 500 nm thick silicon oxide (LOCOS) was deposited for electrical insulation. A 50 nm thick chromium-gold plating base sputtered on the oxide served as galvanic electrode in the deposition process lateron. Spin-coating with PMMA followed yielding a resist thickness of 250 nm. The resist height has to be matched with the intended line width

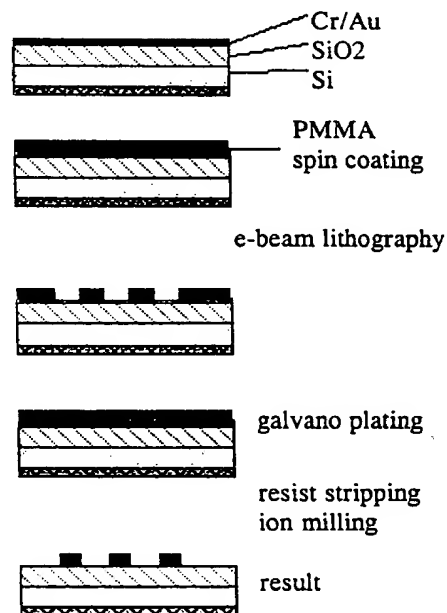


Fig. 2. Scheme of processing of nanometer scale interdigitated electrodes

remaining after development to avoid aspect ratios higher than 5.

As electron-beam-written electrode arrays of this size will reveal line width variations due to the proximity-effect, the geometric data had to be recalculated by the PROXECCO program, multiplying each area by a specific dose correction factor. The resulting data were fractured by a CATS (Transcription Enterprises) processor. Now each electrode array could be written with adequate beam diameter and resolution. A 25 nm spot, which was deflected with 10 nm resolution, has been selected to create the line and space pattern for electrode widths of 50 nm.

After electron exposure the wafers were developed, rinsed, dried and inspected. At this stage some wafers were subjected to line width control measurements to characterize line width loss (Fig. 3). Resist grooves (positive tone development) were measured 20 nm wider than designed. If the exact dimension of a single line would

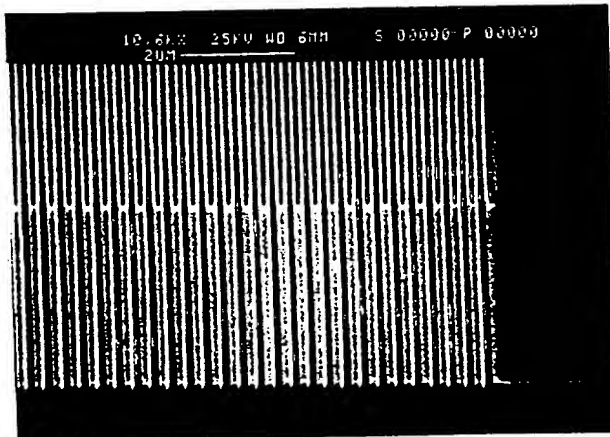


Fig. SEM micrograph of the e-beam structured resist pattern in PMMA as control structure, lines DIT with electrode widths of 250 nm (bright line) are 80 nm, space are 90 nm

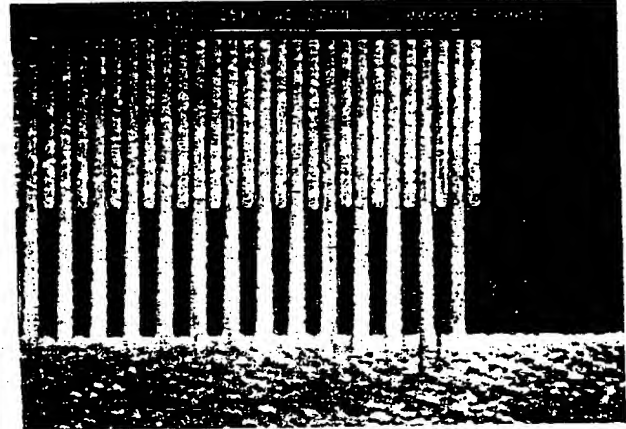


Fig. 4. SEM micrograph of the fabricated gold and gaps of 50 nm

become more important than the periodicity, a revision of the data file prior to the proximity correction must be performed.

Immediately after descumming the pulsed plating of gold (at 2.500 Hz) with a special brightness promoter was performed. A plating speed of 100 nm/min resulted in deposition time of 1 to 2 minutes. To avoid "over-plating" the resist height should exceed the plated features by 50 nm.

For resist stripping an oxygen plasma process was employed, followed by ion-milling of the plating-base which opened the short-cut between the electrodes on the chip. Observing the color of the silicon oxide surface, the endpoint could be detected (Fig. 4).

Only the interdigitated electrode array will be exposed by fluids in the electrochemical cell [1]. An encapsulation with plasma enhanced chemical vapour deposited silicon nitride and optical lithography for opening the active area and connecting pads are the final fabrication steps.

The process scheme was finished by dicing the wafer and bonding the chips onto a support plate.

For the following experiments e-beam fabricated electrode chips, arranged with four pairs of microbands, were used. Each finger electrode is 270 μm long and 50 nm wide. The gaps between the fingers are 100 nm. 600 fingers are connected per band. The separated DIT's were electrically tested for 'shorts' between the band electrodes. Only chips with a resistance between the electrodes of more than 20 GW in the dry state were used.

The electrodes were designed in the way, that all different types have a same electrode area.

3.2. Impedance Measurement

Impedance spectra were taken at a pair of DIT electrodes in order to evaluate the effects of the various steps of preparations and immobilization's. The relations between the interested immittance functions and the permittivity are shown in (1), where are ϵ_0 - dielectric permittivity of free space, A_c - electrode area, l - electrode separation length, Z - impedance, ϵ - dielectric permittivity, c - molar concentration of molar species, m_{pol} - permanent dipole moment, E - electric field

$$\epsilon = \epsilon' - j\epsilon'' = 1 / \{Z j\omega C_c\}, C_c = \epsilon_0 A_c / l, \quad (1a)$$

$$De f - c m_{pol}^4 E^2 \quad (1b)$$

These relations show the dependence between properties of the material and cell geometry's. Additionally, over the complex permittivity and admittance can describe the system in dielectric loss, relaxation, resonance behavior or frequency. With this measuring method is it possible to determine a binding process of biomolecules, when the dipole moment is strong enough to influence the permittivity.

4. Results and discussion

The gold electrodes were modified with a self assembled monolayer consisting of thiol-biotin (Boehringer). The kinetics of the adsorption of 10 mM/l thiol-biotin could be followed by measuring the change of the capacitance of one interdigitated electrode pair with 50 nm width and 100 nm gap (Fig. 5a). After rinsing, 25 μ l avidin-ferritin (sigma) was inserted in the measuring cell. The av-ferritin coating was monitored by cyclic voltammetry to inspect the density of the adsorbed layer in 0.1M/l NaPBS. The adsorption of the av-ferritin resulted in a reversible behavior with $E_{1/2} = 298$ mV / Ag/AgCl and $DE_p = 114$ mV / Ag/AgCl. The peak currents, resulting by the quasi reversible electrochemistry of immobilized ferritin, were calculated for both

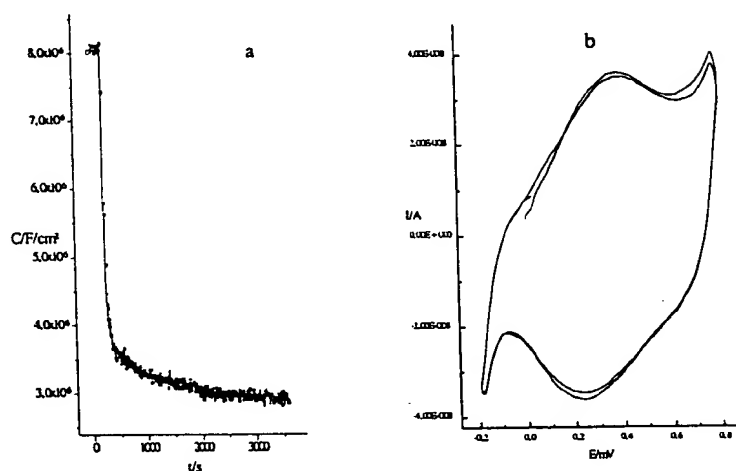


Fig. 5 Electrode modification, a) capacitance time behavior at a pair of gold DIT during the adsorption of the thiol-biotin, b) cyclic voltammogram at an avidin-ferritin modified electrode, scan rate 50 mV/s in 0.1mM NaPBS

peaks as app. 120 nC/electrode (corresponding to $18 \mu\text{C}/\text{mm}^2$).

The influence of the biotin modifier followed by the ferritin immobilization at interdigitated electrode pairs with different geometry's was studied. For these investigation the multi-array-chips were modified and the interdigitated pairs are measured serially in 0.1 M sodium phosphate buffer solution (NaPBS) with and without 2 mM hexacyanoferrate, which have served as redox couple. The analysis of these measurements resulted in a equal behavior for all pairs with different geometry's. This can be explained with the high resistivity of the arrangement, the low influence of the changeable cell constant and the equal electrode areas. Furthermore, an influence of varied buffer solution could not observed.

After the avidin-ferritin immobilization the impedance measurement were repeated. A decrease of the impedance number with decreasing electrode dimensions could be observed. The decrease of the complex impedance for a frequency of 100 mHz is shown in Fig. 6. This shift to lower resistance's over two decades results in a dramatic increase of the signal to noise ratio and the sensitivity.

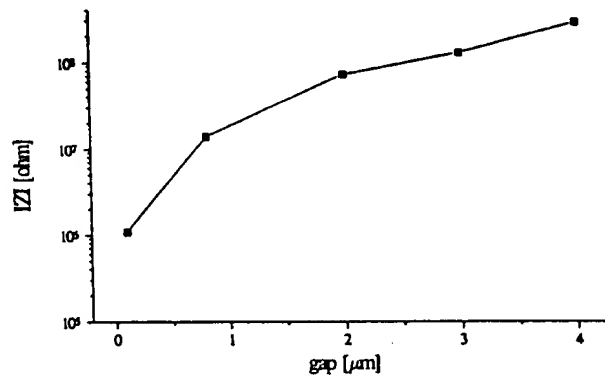


Fig. 6 Complex impedance vs. gaps of avidin-ferritin modified electrodes are shown

The permittivity as function of the applied electric field is describe with the relation (1b), where the permittivity is governed by second order of the field strength. It would be interesting to investigate a DIT at different values of the applied field. For this purpose we used the nanometer scale DIT (50 nm line, 100 nm space) and a applied dc-potential between 0 and 600 mV. The permittivity for thiol-biotin modified electrodes was investigated at first. Not any differences for voltage shifts and frequencies could be found, whereas for the avidin-ferritin layer a shift of the permittivity could be observed (Fig. 7). For the measured values of the permittivity a reference capacity $C_e = 10^{-9}$ F was assumed, corresponding to the theoretical capacitance of the DIT. The complex permittivity as function of the electric field strength is shown in Fig. 7. A quadratic behavior of the applied bias was revealed. The filled symbols are the experimental points and the curve is the fitted values of the theoretical model, which based on the relation (1b).

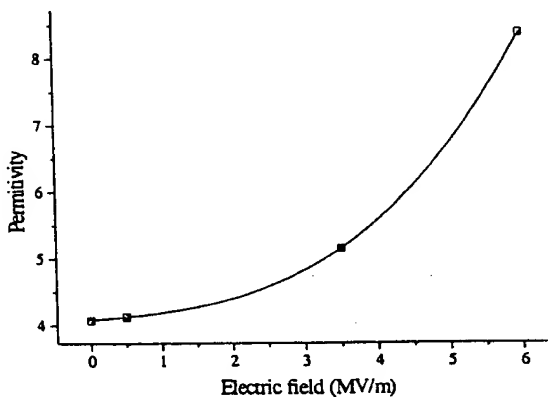


Fig. 7 Plot of the obtained values of the complex permittivity as function of applied electric field for minimum frequency 11 Hz,

Conclusions

An array of closely spaced interdigitated microelectrodes in micro- and nanometer scale was applied to the detection of molecules by surface interactions. The measurement is based on the impedance spectroscopy on a pair of surface modified gold electrodes to detect the change of permittivity. The result show a novel approach for direct detection of affinity binding without any labeling. Combining the functionalized surfaces of nm-DIT a novel and sensitive detection method for the specific interactions of biomolecules is presented. This cross-disciplinary work of nanoengineering, microsystem technology, biochemistry and electronics should show, that new field of applications for biosensing, structural biology or information technologies will be possible.

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